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# The successful use of a submersible ultrasound to confirm pregnancy on free swimming bull sharks, *Carcharhinus leucas*, in a provisioned shark site

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A prototype, fully submersible, high definition ultrasound was used to determine the reproductive state of wild, free-swimming bull sharks, *Carcharhinus leucas*, at a provisioned shark diving site in Playa del Carmen, Mexico. During two opportunistic dives, the presence of embryos was confirmed in three female sharks (greater than 2.0 m total length) and emphasizes the importance of developing and linking emerging technologies with shark diving sites for the conservation of elasmobranch species.

## KEYWORDS

emerging technologies, gestation ground, conservation, management, ecotourism

## 1 Introduction

Diving with sharks has become popular throughout the world in recent decades (Gallagher and Hammerschlag, 2011) and in Mexico has been suggested to contribute approximately 12.4 million in United States dollars (USD) per year to local economies (Cisneros-Montemayor et al., 2013). In addition to an economic importance, observational studies at such shark tourism sites can also provide fishery-independent scientific information to improve population level assessments for certain species (Clua et al., 2010), as not only do these sharks often form seasonal aggregations, they also can be individually identified (Photo-ID; Pierce et al., 2018) and potentially monitored over multiple years. The next stage in the evolution of linking this type of tourism to scientific data collection, is the ability to characterize life history stages to the observed animals.

One particular life history stage that is critical for establishing site-based conservation strategies, such as marine protected areas or time/area closures is an understanding of

where gravid sharks spend their time gestating/and or give birth (Chapman et al., 2013; Sulikowski et al., 2016). However, historically, such reproductive data was collected from sacrificed animals from fisheries, which is problematic for species that have been classified as threatened and in the case of shark diving tourism, can also have a negative socioeconomic impact (Hammerschlag and Sulikowski, 2011). Thus, new approaches to studying elasmobranchs increasingly include non-lethal sampling methods (Penfold and Wyffels, 2019). For example, the use of ultrasounds and short-term restraint for biological sampling has continued to advance this field of research in both wild caught and aquarium elasmobranchs (Carrier et al., 2003; Sulikowski et al., 2016, Murakumo et al., 2020). However, the use of ultrasound technology on free swimming sharks in the wild, has only been documented on one species (Murakumo et al., 2020), and has yet to be applied to sharks aggregating at dive sites.

To fill this knowledge gap, a prototype, submersible, high definition ultrasound developed by EI Medical (Inc.) was field tested on free swimming bull sharks, *Carcharhinus leucas*, within a provisioned dive site in the coast of Playa Del Carmen to determine if any of sharks were gravid at this location.

## 2 Materials and methods

Opportunistic sampling occurred during two, 20 min dives, approximately 1 km of the coast of Playa del Carmen, in Quintana Roo state, Mexico (Figure 1). Water depth was 24 m and the substrate comprised of sandy bottom. A fully submersible ultrasonography was performed on the abdominal region of free-swimming female bull sharks to assess reproductive stage (Figure 2). Here, a topside Ibex EVO III portable ultrasound (E.I. Medical Imaging) was redesigned and encased in waterproof housing (via proprietary specifications) for use at depths greater than 30 meters, herein termed the Aquanaut (Figure 3). The Aquanaut was equipped with a 60 mm curved linear array 2.5 to 5 MHz transducer (model eCL3) capable of a 30 cm scan depth which was connected to the unit with a 3 m cord. The Aquanaut recorded one continuous video until the dive ended. Bull sharks were fed by a professional shark handler with chunked, local fish species, and led

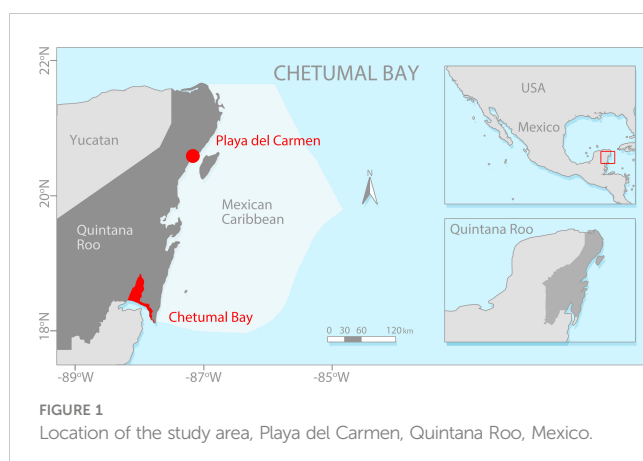
single file so that the transducer could make contact as the sharks slowly swam by (Figure 2). Due to limited bottom dive time (20 min), the largest sharks estimated visually, were targeted for ultrasonography. In addition, due to the frequency of dives performed (two to three per day for four to five months over the course of a dive season) individual bull sharks were able to be identified by the dive operators. Once targeted, a second diver recorded a video of the scanning event for each shark as it moved away from the handler. Each video recording of the scanning event was time stamped and later coordinated with the internal clock on the ultrasound to link an image of the gravid female to her recorded embryos. Scanning was performed primarily on the lateral surface from the pectoral to the pelvic fin in either transverse or longitudinal orientation to obtain cross sectional and lengthwise images, respectively (Figure 2). Each scan lasted between one to three seconds. Collected video saved on the Aquanaut were used to create still images of observed embryos (Sulikowski and Hammerschlag, 2023). Stills from the video were then used to measure (via proprietary software pre-installed on the Aquanaut) pup diameter (cm) along the transverse axis (Sulikowski et al., 2016).

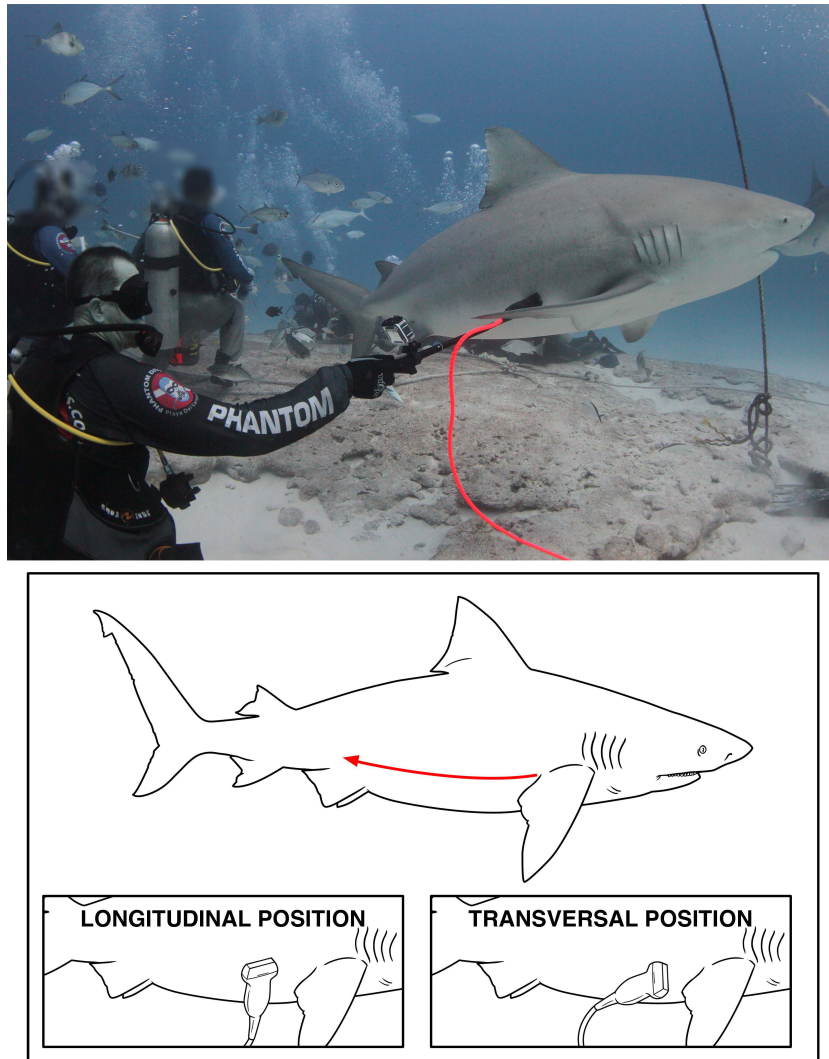
## 3 Results

Over the course of the two 20 minute dives, individual sharks made several passes and based on the analysis of the Aquanaut video, three sharks were identified to be gravid (Figure 4) by the presence of identifiable embryos within the uterus (Sulikowski and Hammerschlag, 2023). Proprietary software measurements from still images indicated the embryos measured were approximately 4 cm in circumference. While the exact location and orientation of the embryos could not be determined, given the short duration of transducer shark contact, the observed sizes are similar to those reported from a gravid female captured on November 22nd-2022 by local fishermen in Isla Mujeres, Quintana Roo (a nearby location to Playa del Carmen), during a fishing survey realized by Save Our Sharks staff.

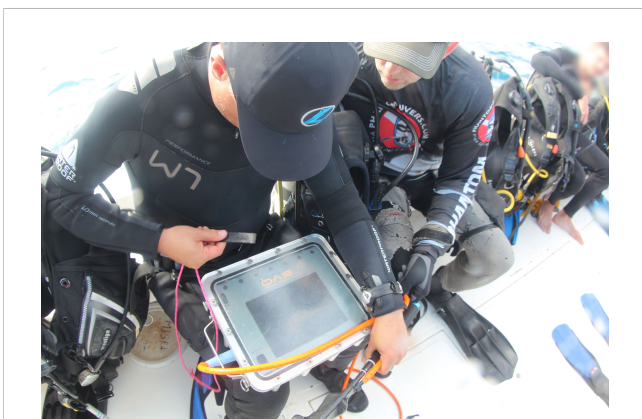
## 4 Discussion

The use of ultrasonography as a non-invasive methodology has proven to be a useful tool for the study of reproductive aspects in both captive and wild caught sharks (Walsh et al., 1993; Daly et al., 2007; Sulikowski et al., 2016; Inoue et al., 2022; Santos et al., 2022; Sulikowski and Hammerschlag, 2023). However, the use of this technology adapted for use underwater on free swimming wild animals is scant, with only one published study to date on whale sharks (Matsumoto et al., 2023). The study herein adds to this limited body of work and shows the utility of this technology to identify gravid females in a provisioned dive site. The positive outcome has several implications for the preservation of specific life history stages for sharks and potentially other elasmobranchs. For example, it is well established that critical component of successful conservation of wild populations is an understanding of a species'

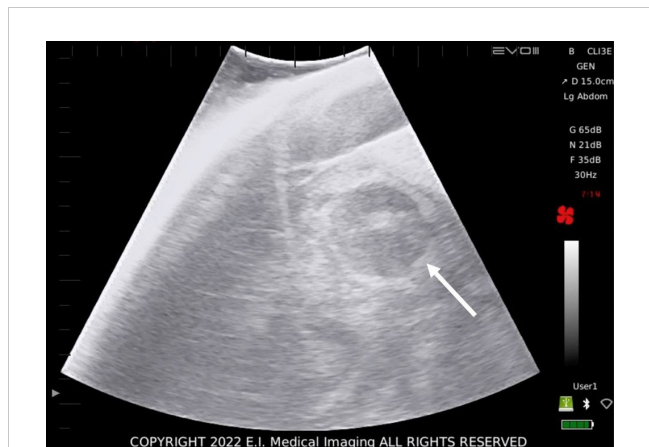




**FIGURE 2**  
 Representative distance from and lateral scanning of a pregnant bull shark. The Aquanaut underwater ultrasound was connected to 60 mm curved linear array 2.5 to 5 MHz transducer. Scanning produced either a cross section or lengthwise orientation of embryos within the uterus.



**FIGURE 3**  
 Topside Ibex EVO III Aquanaut portable ultrasound (EI Medical Imaging) encased in waterproof housing.



**FIGURE 4**  
 Representative transverse ultrasound images of bull shark *Carcharhinus leucas* obtained from gravid females with identified embryos *in utero*. Arrows point to the embryo.

reproductive biology (Natanson et al., 2019) as it can assist in stock assessments thereby informing management decisions related to protected area designation (Awruch, 2013).

The identification of pregnant bull sharks in Playa del Carmen, suggests that this area may serve as a gestation ground and as such provides critical habitat for this life history stage. A similar phenomenon has also been observed in tiger sharks within Tiger Beach, a provisioned dive site within Bahamas (Sulikowski et al., 2016). Anecdotal evidence from Playa del Carmen dive operations suggest female bull sharks regularly migrate into the shallow waters of the Playa del Carmen beginning in November and stay until March when water temperatures are above 26°C (78.8°F). Bull sharks are absent from this area by April when waters are well above 26°C. The absence of bull sharks by April suggests that similar to tiger sharks at Tiger Beach (Sulikowski et al., 2016), environmental or ecological drivers are responsible for the observed movement out of the dive site (Chapman et al., 2013). For pregnant females observed herein, seeking a suitable habitat for parturition provides a likely explanation (Chapman et al., 2013). For example, Blanco-Parra et al. (2022) have recently demonstrated that Chetumal Bay (Figure 1) serves as a nursery area for bull sharks in the Mexican Caribbean and reported the presence of neonates beginning in May.

Whether the gravid bull sharks analyzed in this study travel to Chetumal Bay (approximately 360 km from Playa del Carmen) requires further investigation *via* other methodologies (Sulikowski and Hammerschlag, 2023). Regardless, the results of the current study emphasize the importance of developing and linking emerging technologies with ecotourist sites for the conservation of elasmobranch species.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was reviewed and approved by ASU Protocol Number: 23-1955R Protocol Title: Using innovative and non-lethal techniques to study the biology, ecology, and habitat utilization of sharks Institutional Animal Care and Use Committee (IACUC) Office of Research Integrity and Assurance Arizona State University.

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## Author contributions

EH-P and JS conceptualized the study. EH-P, IC-S, JL-C and JS contributed for the data collection and field work. EH-P, JL-C and JS look for funding acquisition. JS performed data analyses. EH-P, IC-S, JS wrote the original draft of the manuscript. All the authors reviewed and edited the manuscript. All the authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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